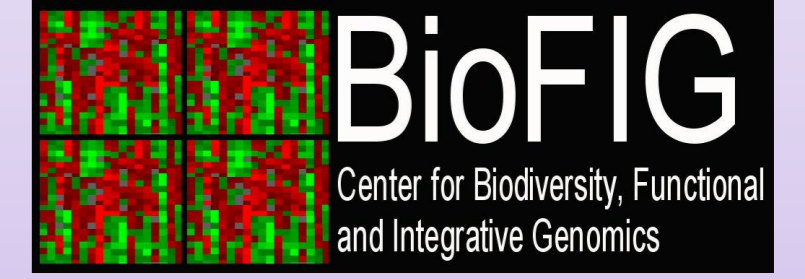


Rac1b expression reverts B-Raf-V600E-induced senescence in colorectal cells



Andreia Henriques^{1,2} Patrícia Barros^{1,2} Paulo Matos^{2,3} and Peter Jordan^{1,2}

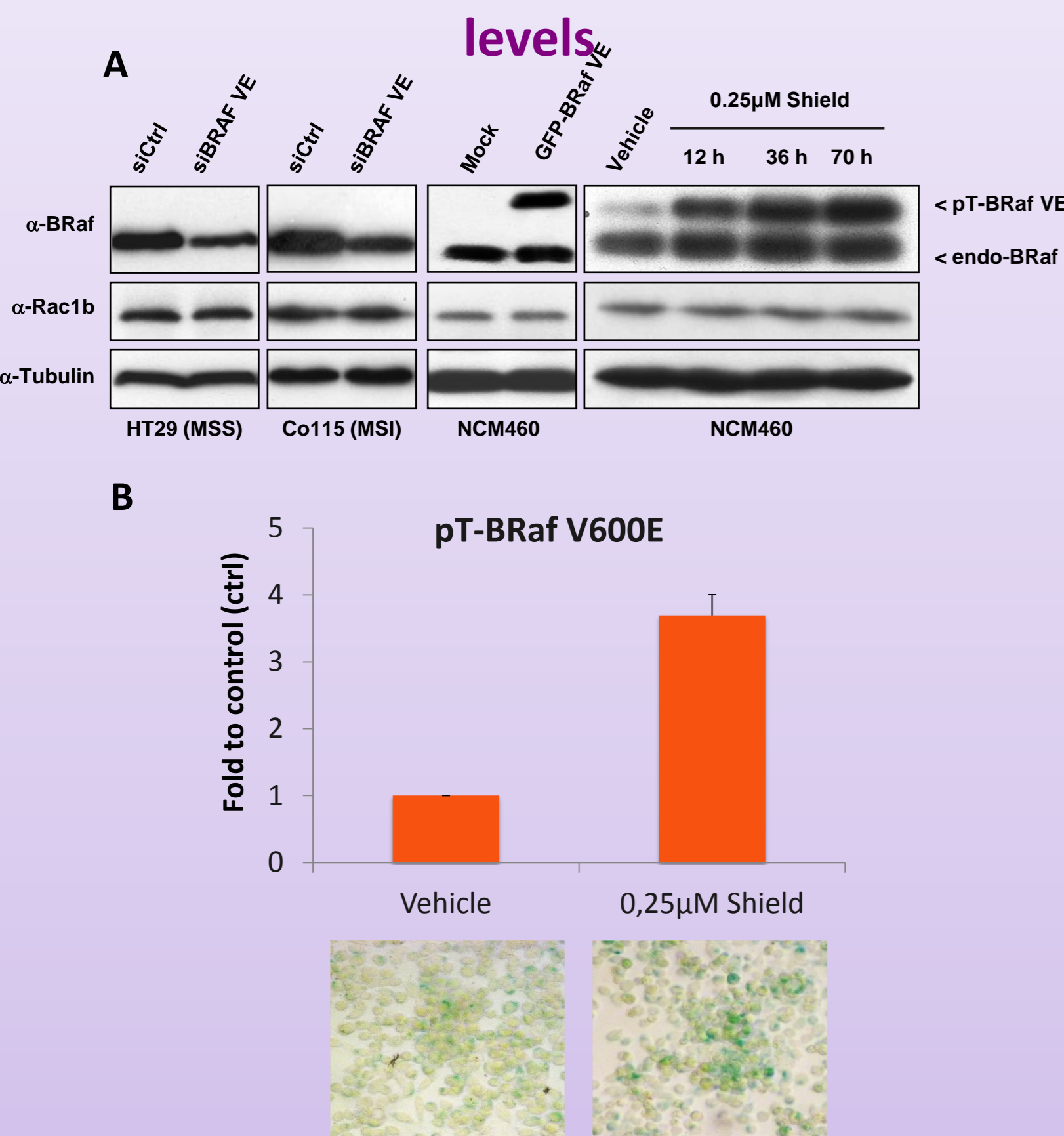


¹ Department of Human Genetics, National Health Institute Doutor Ricardo Jorge, Lisbon, Portugal;
² BioFIG - Centre for Biodiversity, Functional and Integrative Genomics, Faculty of Sciences, University of Lisbon;
³ Department of Chemistry and Biochemistry, Faculty of Sciences, University of Lisbon.

Summary

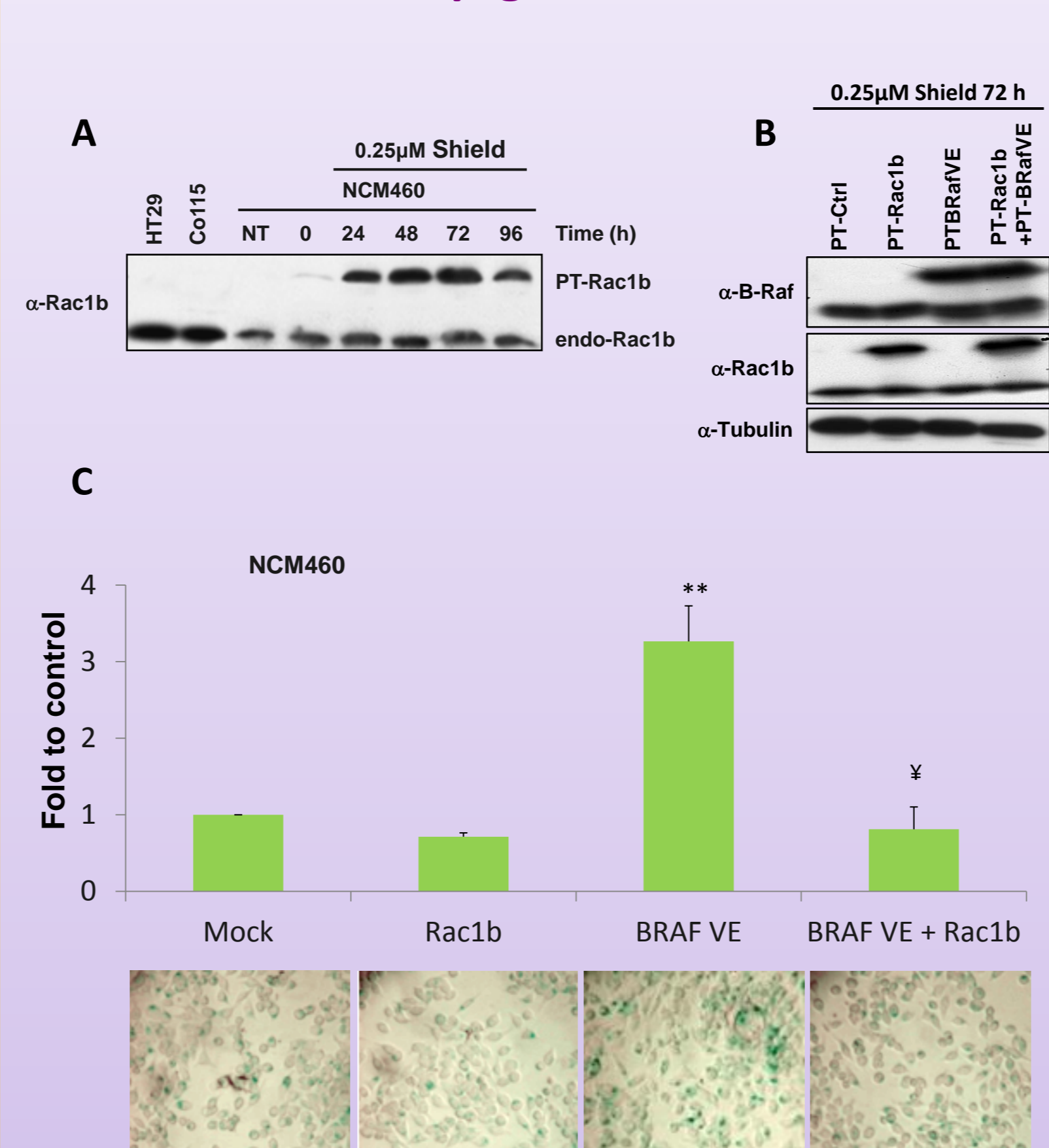
Mutations in the *BRAF* oncogene have been identified as a tumor-initiating genetic event in mainly melanoma, thyroid and colon cancer, resulting in an initial proliferative stimulus that is followed by a growth arrest period known as oncogene-induced senescence (OIS). It remains unknown what triggers subsequent escape from OIS to allow further tumor progression. A previous analysis revealed that overexpression of splice variant Rac1b occurs in around 80% of colorectal tumors carrying a mutation in *BRAF*. Using both BRaf-V600E-directed RNAi and overexpression we demonstrate that this mutation does not directly lead to Rac1b overexpression, indicating the latter as an independent event during tumor progression. Nonetheless, we observed that expression of oncogenic BRaf-V600E in non-transformed colonocytes (NCM460 cell line) increased both the transcript and protein levels of p14^{ARF}, p15^{INK4b} and p21^{CIP1} and led to increased expression of β -galactosidase, all indicators of OIS induction. Interestingly, whereas the protein levels of these markers were reduced upon Rac1b overexpression, the levels of their respective transcripts remained unchanged. Importantly, the co-expression of Rac1b with B-Raf-V600E reverted the OIS phenotype, reducing the expression levels of the cell-cycle inhibitors and β -galactosidase to those of control cells. These data identify increased Rac1b expression as one potential mechanism by which colorectal tumor cells can escape from B-Raf-induced OIS.

Expression of B-Raf-V600E in non-transformed colonocytes induces cellular senescence but does not alter Rac1b levels



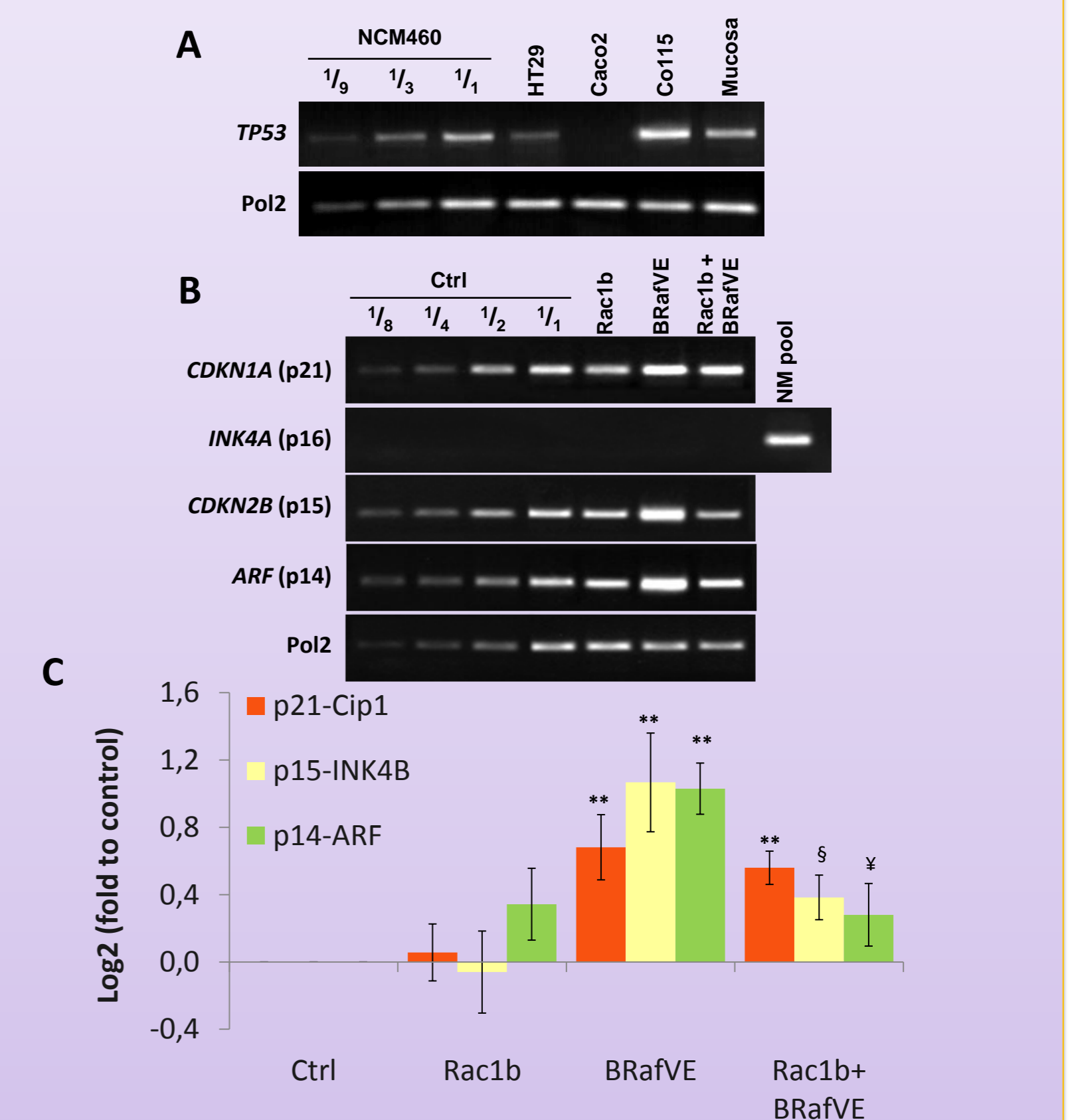
(A) Colon cancer cells HT29 and Co115, transfected with either B-Raf-V600E-specific or control siRNA oligonucleotides. Following 48 h, the resulting expression levels of B-Raf and Rac1b proteins were analyzed by Western blot. Note that specific reduction in the accumulation of the oncogenic B-Raf variant had no effect on Rac1b levels. NCM460 transfected with either a GFP-B-Raf-V600E vector for 48 h or with an inducible ProteoTuner (pT)-B-Raf-V600E fusion protein. Again, no changes in Rac1b protein levels were observed after the indicated incubation periods. (B) Senescence-associated β -galactosidase (SA- β -gal) activity observed in NCM460 cells transfected with pT-B-Raf-V600E following 70 h of incubation with control solvent or Shield1 for transgene induction.

Rac1b co-expression abrogates B-Raf-V600E-induced SA- β -gal accumulation

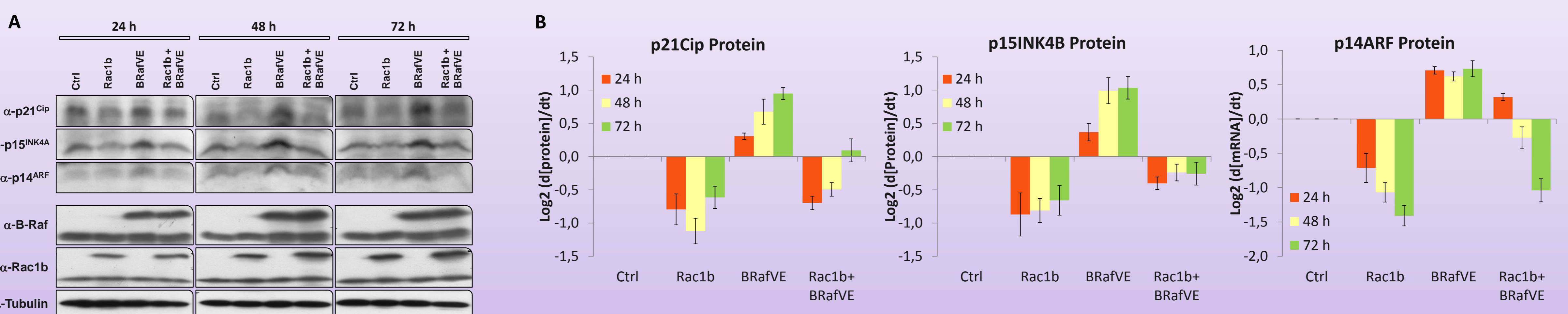


(A) NCM460 cells were transfected with inducible ProteoTuner (pT)-Rac1b construct and Rac1b expression was induced for 96 h. Cells maintained Rac1b expression levels comparable to those of the cancer cell lines HT29 and Co115 for 72 h. (B) Western blot showing that individual or combined expression of PT-B-Raf-V600E and PT-Rac1b in NCM460 cells reached equivalent fusion protein levels after 72 h of induction with Shield. (C) Senescence-associated β -galactosidase (SA- β -gal) activity observed in NCM460 cells under the transfection conditions described in (B). Note that co-expression of Rac1b abrogates the B-Raf-V600E induced senescence activity. Statistically significant differences ($p < 0.05$) of data from Mock versus BRafVE are indicated as ** and for BRafVE versus BRafVE+Rac1b as $\$$.

B-Raf-V600E but not Rac1b increase the expression of OIS-associated cell-cycle inhibitor genes



Rac1b overexpression antagonizes B-Raf-V600E-induced cell cycle inhibitor expression at the protein level



NCM460 cells were transfected with the indicated pT-based inducible constructs and lysed after 24, 48 and 72 h of Shield treatment. (A) Samples were analyzed by SDS-PAGE and Western blot using the indicated antibodies. (B) Densitometric analysis of band intensities to quantify the effects of B-Raf-V600E and Rac1b at the indicated time point (dt) on the indicated concentration of cell cycle-inhibitory proteins (d[protein]). Note that the p14, p15 and p21 protein levels were significantly downregulated by Rac1b overexpression.

Conclusions:

- In a normal colonocyte model, the expression of oncogenic mutant B-Raf-V600E leads to oncogene-induced senescence.
- Co-expression of Rac1b counteracts this phenotype by suppressing the upregulation of p14^{ARF}, p15^{INK4b} and p21^{CIP1}.
- Overexpression of Rac1b, which correlates with mutation in *BRAF* in colorectal tumors, is proposed as a mechanism to escape from OIS.